

# Application of Hectorite-Coated Silica Gel Particles as a Packing Material for Chromatographic Resolution

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ABSTRACT: A new type of clay column particles was prepared, in which a hectorite layer (c.a. 0.1  $\mu\text{m}$  thickness) covered uniformly the surface of amorphous silica particles with an average radius of 5  $\mu\text{m}$  (ref. Okada, T. et al., *The Journal of Physical Chemistry C*, **116**, 21864-21869 (2012)). The hectorite layer was fully ion-exchanged with  $\Delta$ -[Ru(phen)<sub>3</sub>]<sup>2+</sup> (phen = 1,10-phenanthroline) ions by being immersed in a methanol solution of  $\Delta$ -[Ru(phen)<sub>3</sub>](ClO<sub>4</sub>)<sub>2</sub> (1 mM). The modified silica gel particles thus prepared were packed into a stainless steel tube (4 mm (i.d.) x 25 cm) as a HPLC column. Optical resolution was achieved when the racemic mixtures of several metal complexes or organic molecules were eluted with methanol. In case of tris(acetylacetonato)ruthenium(III) ([Ru(acac)<sub>3</sub>]), for example, the  $\Lambda$ - and  $\Delta$ -enantiomers gave an elution volume of 2.6 mL and 3.0 mL respectively, with the separation factor 1.2. The total elution volume (5 mL) was nearly one-tenth for the previously reported column of the same size (RU-1 (Shiseido Co. Ltd)) packed with the spray-dried particles of synthetic hectorite (average radius 5  $\mu\text{m}$ ) ion-exchanged by the same Ru(II) complexes.

## Introduction

The production of chiral molecules often constitutes a crucial step in synthetic chemistry<sup>1,2</sup>. Today high performance liquid chromatography (HPLC) plays a central role in obtaining optically pure materials from racemic mixtures. A variety of chiral HPLC columns has been developed and exhibit their characteristic features in practical application<sup>3-14</sup>.

A chiral column based on layered clay minerals (denoted as RU-1 (Shiseido Co. Ltd)) has been reported<sup>15-18</sup>. An ion-exchanged adduct of a clay and enantiomeric tris(1,10-phenanthroline)ruthenium(II) ion ( $\Delta$ - or  $\Lambda$ -[Ru(phen)<sub>3</sub>]<sup>2+</sup>) was used as a packing material. A chiral molecule in a mobile phase is adsorbed on a clay surface stereoselectively<sup>19-23</sup>. The complex ([Ru(phen)<sub>3</sub>]<sup>2+</sup>) took uniform orientation on a two-dimensional network structure of phyllosilicate surface. A vacant space surrounded by enantiopure [Ru(phen)<sub>3</sub>]<sup>2+</sup> molecules provides an adsorption site with high chiral discrimination<sup>24</sup>.

In case of RU-1, a packing material was prepared by ion-exchanging the spray-dried particles of a synthetic hectorite (average radius 5  $\mu$ m) with  $\Delta$ - or  $\Lambda$ -[Ru(phen)<sub>3</sub>]<sup>2+</sup> cations<sup>15</sup>. The column demonstrated wide application for resolving both organic and inorganic compounds<sup>25-27</sup>. The clay column, however, adsorbed molecules so strongly that a large volume of solvent was required for elution. In case of tris(acetylacetonato)ruthenium(III) ([Ru(acac)<sub>3</sub>]), for example, the total elution volume was 100 mL when the compound was mounted and eluted with methanol on a 4 mm (i.d.) x 25 cm column. The volume is nearly ten times larger than other commercial ones of the same size such as a column coated with derivatized cellulose (Daicel, Japan).

In the present study, a new type of clay column was prepared by using a silica gel particle coated with a thin film (ca. 0.1  $\mu$ m thickness) of a hectorite-like layered silicate (abbreviated as Hect). Hence, coating with the Hect was conducted through the heterogeneous nucleation

reaction in an aqueous solution of Li and Mg salts on the surface of a silica particle.<sup>28-30</sup> An inner silica part was covered with an outer thin layer of Hect. If the adsorption-desorption equilibrium is accomplished within such a thin layer, elution would proceed rapidly, maintaining high degree of chiral discrimination. Motivated by this expectation, a clay column based on a Hect silica gel was prepared and its chromatographic behavior was investigated.

## Experimental

### *Materials*

Lithium fluoride, magnesium dichloride hexahydrate, and urea, dioctadecyldimethylammonium (abbreviated to 2C<sub>18</sub>) bromide were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Silica gel powder with average radius of 5 μm (Li-Chromosorb, Merck KGaA Co., Ltd. (Darmstadt, Germany) was used as the source of hectorite. All these chemicals were used without further purification. The synthesis and resolution of [Ru(phen)<sub>3</sub>](ClO<sub>4</sub>)<sub>2</sub> was performed according to the reported method<sup>19</sup>. [M(acac)<sub>3</sub>] (M = Ru, Co, Ir and Rh) and 1,1'-binaphthol were used as purchased (Aldrich). [Ru(acac)<sub>2</sub>(C<sub>10</sub>-dbm)] (C<sub>10</sub>-dbm = 1,3-(4-*n*-decyloxyphenyl)-1,3-propanedionato) and [{Ru(acac)<sub>2</sub>}<sub>2</sub>(baet)] (baet: 1,2-diacetyl-1,2-dibenzoyl-ethanate) were synthesized as reported elsewhere<sup>25,31</sup>.

### *Preparation of Hect-coated silica gel particles*

Coating of silica gel particles with a thin film of hectorite was carried out based on our recent report<sup>29</sup>. The molar ratio of LiF:MgCl<sub>2</sub>:SiO<sub>2</sub>:urea was 0.21:0.8:8.0:8.0 in the starting mixture, where the amounts of Li and Mg sources relative to the amount of SiO<sub>2</sub> were decreased by 15% from the reported ratio of Li: Mg: Si of 1.4:5.6:8.0<sup>32,33</sup>. In the experiment, urea (2.16 g), MgCl<sub>2</sub>·6H<sub>2</sub>O (0.73 g) and LiF (0.024 g) were dissolved in water (80 mL). The resulting solution was

mixed with an aqueous suspension of spherical silica particles (2.16 g in 20 mL of water) using a mechanical homogenizer (at 4.6k rpm) for 30 min at room temperature. The slurry was transferred to a Teflon-lined autoclave and heated to 373 K for 48 h. The autoclave was rotated at 15 rpm using a hydrothermal synthesis reactor unit (Hiro Company) during the heat treatment. The slurry was then cooled in an ice bath and centrifuged (at 1400 g for 20 min). Thereafter the precipitate was collected and dried at 323 K.

The cation-exchange capacity (CEC) of the Hect-coated silica particles was determined using the calcium-exchanged form; the Ca-exchanged form was prepared by immersing the sample (1.0 g) into an aqueous solution of calcium dichloride at a concentration (10 mM, 50 mL), which was 10-fold in excess of the estimated CEC (0.20 mEq/g sample). The solution was renewed twice and used after washing with deionized water until negative  $\text{Cl}^-$  test was obtained. The Ca-exchanged form (0.1 g) was repeatedly washed with an aqueous solution of NaCl (4 mM, 25 mL). The resulting supernatant was used for determining the concentration of calcium ions using ion chromatography.

#### *Preparation of a column*

$\Delta\text{-[Ru(phen)}_3\text{](ClO}_4\text{)}_2$  in methanol solution (1.1 mM, 28 mL) was allowed to react with the Hect-cated silica sample (3 g) under magnetically stirring at room temperature for ca. 1 hour, renewing the solutions twice. The product was collected by filtration and washed with methanol (ca. 10 mL). The resulting slurry was poured into a 4 mm (i.d.) x 25 cm stainless steel column, and was packed using a continuous flow of methanol ( $1 \text{ mL min}^{-1}$ ). The column was abbreviated to CSP-1, hereafter.

#### *Equipments*

HPLC was carried out with a chromatograph (a JASCO a HPLC 980 system with a UV-975

detector). The elution was detected by electronic absorption spectra. The circular dichroism spectra of the eluted fractions were recorded with a polarimeter J-500 (Jasco, Japan). X-Ray powder diffraction (XRD) patterns were obtained by a Rigaku RINT 2200V/PC diffractometer (monochromatic Cu K $\alpha$  radiation), operated at 20 mA, 40 kV. Scanning electron micrographic (SEM) images were captured on a Hitachi SU-8000 field-emission scanning electron microscope (operated at 1 kV) after osmium plasma coating.

## Results

### *Characterization of Hect-coated silica gel particles*

The SEM images of a Hect-coated silica gel sample and pristine silica gel particles are shown in Fig. 1. The surfaces of the silica particles were smooth (Fig. 1a) but the surfaces of the Hect-coated particles were rough with fine plate-like particles (Fig. 1b). The surface of the silica gel was thoroughly and homogeneously covered with the fine plate-like particles. The powder XRD pattern of the Hect-coated sample (Fig. 1b) showed reflection peaks that were ascribed to hectorite at 35° ( $2\theta$  Cu K $\alpha$ ) for (130) and 61° ( $2\theta$ ) for (060), with the halo pattern of amorphous silica in the range of 20–30° ( $2\theta$ ). The plate-like fine particles observed were therefore regarded as hectorite-like layered silicate (Hect) crystals. Diffraction peak that could be ascribed to the (001) plane did not appear, meaning that the Hect grown was poor crystalline, especially parallel to the  $c$ -axis. CEC was determined using the Ca<sup>2+</sup>-exchanged form to be 0.086 meq/g. Because the molar ratio in the starting mixture was Li:Mg:Si = 0.21:0.80:8.0, CEC of the Hect crystals in the sample was estimated to be 0.5–0.6 mmol/g Hect<sup>32</sup>. Assuming that the crystal layer of Hect homogeneously covered the surface of the silica gel (spherical  $\phi$  = 5  $\mu$ m in average), the estimated thickness of the Hect layer was 0.12 ~ 0.15  $\mu$ m. After the cation-

exchange reactions with  $\Delta$ -[Ru(phen)<sub>3</sub>]<sup>2+</sup> cations to the saturated amount of the CEC, the resulting slurry was used for the chromatographic resolution tests.

**(Insert Fig. 1)**

### *Chromatographic resolution*

Fig. 2 shows the chromatogram when 0.1  $\mu$ mol of [Ru(acac)<sub>3</sub>] was eluted with methanol on CSP-1 at a flow rate of 1.0 mL min<sup>-1</sup>. Two sharp peaks with slightly overlapping were obtained at an elution volume of 2.6 mL and 3.0 mL, respectively. From the circular dichroism (CD) spectrum of the fraction collected at each peak, the first and second peaks contained the excess  $\Lambda$ - and  $\Delta$ -enantiomers, respectively. Fig. 2b shows the chromatogram when the same molecule was mounted on the known column packed with the spray-dried hectorite particles (RU-1) ion-exchanged with  $\Delta$ -[Ru(phen)<sub>3</sub>]<sup>2+</sup> (experimental section) and eluted under the same conditions. Two separated peaks were obtained with the elution volumes of 19.1 mL and 49.2 mL, respectively. From the CD spectra of the collected fractions, the same enantiomeric order as in Fig. 2a was confirmed.

**(Insert Fig. 2)**

As shown in Fig. 3, the separation factor stayed nearly constant for the change of a flow rate from 0.25 mL min<sup>-1</sup> to 1 mL min<sup>-1</sup>. Thus the complete elution finished within 5 min. The effects of the mounted amount were studied as shown in Fig. 3. According to the results, the maximum

capacity of the present column (CSP-1) was estimated to be  $10^{-6}$  mole per one column. This was ca. 1% of the maximum capacity of RU-1.

**(Insert Fig. 3)**

Fig. 4 shows the chromatogram when a racemic mixture of 1,1'-binaphthol was eluted with methanol. Although two peaks were appreciably overlapped, the CD spectra of the collected fractions confirmed that the compound was separated into *S*- (first peak) and *R*- (second peak) enantiomers, respectively.

**(Insert Fig. 4)**

In Table I, the results of resolution for a number of organic and inorganic compounds (the chromatograms are shown in Supplementary material, Figure S1) are summarized. In all cases, the required elution volume was nearly 10 % of the previous column (RU-1).

**(Insert Table I)**

## **Discussion**

In the present study, the chromatographic behavior was compared between two clay columns: the one used spray dried particles of synthetic hectorite (denoted as RU-1) and the other is the silica gel particles coated with a thin film of Hect (denoted as CSP-1). In both materials, enantiopure  $[\text{Ru}(\text{phen})_3]^{2+}$  ions were ion-exchanged with hectorite as a chiral modifier.



The same mechanism was concluded to operate in chiral discrimination since they always gave the same elution order of resolved enantiomers (Table 14). In other words, an injected molecule was associated with enantiopure  $[\text{Ru}(\text{phen})_3]^{2+}$  stereoselectively either on the external surface or in the interlayer space of a clay mineral. According to Table 14, a large difference was observed between these columns for the elution volume, or the elution volume on the present column was nearly one-tenth of that of RU-1. In case of RU-1, a resolved molecule penetrated the whole part of the particles. Accordingly the elimination process from the inner part of particles to a mobile phase took much time. In case of CSP-1, a resolved molecule was adsorbed in a thin layer of Hect. Therefore adsorption-dissociation equilibration was attained rapidly. From the practical point of view, the shortening of elution time is important for saving time and eluting solvents.

As for the elution behavior of resolved species, some tailing in each peak was observed in CSP-1, which reduced the efficiency of resolution. Most probably the tailing was caused by the non-uniform nature of a hectorite layer coating a silica gel as deduced from the SEM image (Figure 1a). More strictly uniform formation of Hect layer is now under progress.

## **Conclusion**

Silica (inner)-Hect (outer) hybrid particles were prepared and applied as a packing material for chromatographic resolution. The surface layer of Hect was ion-exchanged with a chiral metal complex,  $\Delta\text{-}[\text{Ru}(\text{phen})_3]^{2+}$ . When the modified particle was used as a packing material, a number of racemic mixtures including chiral neutral metal complexes and 1,1'-binaphthol were optically resolved. Elution volume was reduced to 10% when it was compared with the previously reported column that used spray-dried hectorite particles.

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## Figure captions

**Fig. 1** The SEM images of (a) pristine silica gel particles and (b) Hect-coated silica gel sample, (c) XRD pattern of the Hect-coated sample.

**Fig. 2** Chromatograms when a racemic mixture of  $[\text{Ru}(\text{acac})_3]$  was eluted with methanol on columns packed with (a)  $\Delta$ - $[\text{Ru}(\text{phen})_3]^{2+}$ -Hect-coated silica gel and (b)  $\Delta$ - $[\text{Ru}(\text{phen})_3]^{2+}$ -RU-1. The flow rate was  $1 \text{ mL min}^{-1}$ , and the elution was monitored by the absorbance at 346 nm at room temperature.

**Fig. 3** Chromatograms when a racemic mixture of  $[\text{Ru}(\text{acac})_3]$  was eluted with methanol on columns packed with  $\Delta$ - $[\text{Ru}(\text{phen})_3]^{2+}$ -Hect-coated silica gel. The flow rate varied from (a) 1.0 to (b) 0.50 and to (c)  $0.25 \text{ mL min}^{-1}$ , and the elution was monitored by the absorbance at 346 nm at room temperature.

**Fig. 4** (a) Chromatogram when a racemic mixture of 1,1'-binaphthol was eluted with methanol on columns packed with  $\Delta$ - $[\text{Ru}(\text{phen})_3]^{2+}$ -Hect-coated silica gel. The flow rate was  $1 \text{ mL min}^{-1}$ ; (b) circular dichroism spectra of 1,1'-binaphthol in methanol corresponding to the chromatogram.

**Fig. 5** Schematic drawing of a structure of  $\Delta$ - $[\text{Ru}(\text{phen})_3]^{2+}$ -exchanged Hect.